



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2014

Loss-of-function of β -catenin bar-1 slows development and activates the Wnt pathway in *Caenorhabditis elegans*

van der Bent, M Leontien ; Sterken, Mark G ; Volkers, Rita J M ; Riksen, Joost A G ; Schmid, Tobias ; Hajnal, Alex ; Kammenga, Jan E ; Snoek, L Basten

Abstract: *C. elegans* is extensively used to study the Wnt-pathway and most of the core-signalling components are known. Four β -catenins are important gene expression regulators in Wnt-signalling. One of these, bar-1, is part of the canonical Wnt-pathway. Together with Wnt effector pop-1, bar-1 forms a transcription activation complex which regulates the transcription of downstream genes. The effects of bar-1 loss-of-function mutations on many phenotypes have been studied well. However, the effects on global gene expression are unknown. Here we report the effects of a loss-of-function mutation bar-1(ga80). By analysing the transcriptome and developmental phenotyping we show that bar-1(ga80) impairs developmental timing. This developmental difference confounds the comparison of the gene expression profile between the mutant and the reference strain. When corrected for this difference it was possible to identify genes that were directly affected by the bar-1 mutation. We show that the Wnt-pathway itself is activated, as well as transcription factors elt-3, pqm-1, mdl-1 and pha-4 and their associated genes. The outcomes imply that this response compensates for the loss of functional bar-1. Altogether we show that bar-1 loss-of function leads to delayed development possibly caused by an induction of a stress response, reflected by daf-16 activated genes.

DOI: <https://doi.org/10.1038/srep04926>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-101314>

Journal Article

Published Version

Originally published at:

van der Bent, M Leontien; Sterken, Mark G; Volkers, Rita J M; Riksen, Joost A G; Schmid, Tobias; Hajnal, Alex; Kammenga, Jan E; Snoek, L Basten (2014). Loss-of-function of β -catenin bar-1 slows development and activates the Wnt pathway in *Caenorhabditis elegans*. *Scientific Reports*, 4(4926):online. DOI: <https://doi.org/10.1038/srep04926>



OPEN

SUBJECT AREAS:
TRANSCRIPTOMICS
GENE EXPRESSIONReceived
27 February 2014Accepted
23 April 2014Published
13 May 2014Correspondence and
requests for materials
should be addressed to
J.E.K. (Jan.
Kammenga@wur.nl) or
L.B.S. (Basten.Snoek@
wur.nl)* These authors
contributed equally to
this paper.Loss-of-function of β -catenin *bar-1* slows
development and activates the Wnt
pathway in *Caenorhabditis elegans*M. Leontien van der Bent^{1*}, Mark G. Sterken^{1*}, Rita J. M. Volkers^{1*}, Joost A. G. Riksen¹, Tobias Schmid²,
Alex Hajnal², Jan E. Kammenga¹ & L. Basten Snoek¹¹Laboratory of Nematology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands, ²Institute of
Molecular Life Sciences, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.

C. elegans is extensively used to study the Wnt-pathway and most of the core-signalling components are known. Four β -catenins are important gene expression regulators in Wnt-signalling. One of these, *bar-1*, is part of the canonical Wnt-pathway. Together with Wnt effector pop-1, *bar-1* forms a transcription activation complex which regulates the transcription of downstream genes. The effects of *bar-1* loss-of-function mutations on many phenotypes have been studied well. However, the effects on global gene expression are unknown. Here we report the effects of a loss-of-function mutation *bar-1(ga80)*. By analysing the transcriptome and developmental phenotyping we show that *bar-1(ga80)* impairs developmental timing. This developmental difference confounds the comparison of the gene expression profile between the mutant and the reference strain. When corrected for this difference it was possible to identify genes that were directly affected by the *bar-1* mutation. We show that the Wnt-pathway itself is activated, as well as transcription factors *elt-3*, *pqm-1*, *mdl-1* and *pha-4* and their associated genes. The outcomes imply that this response compensates for the loss of functional *bar-1*. Altogether we show that *bar-1* loss-of function leads to delayed development possibly caused by an induction of a stress response, reflected by *daf-16* activated genes.

The Wnt/ β -catenin pathway is highly conserved across metazoans and is essential for many cellular functions like cell specialization, cellular migration, adhesion and development. Although a key pathway in invertebrates and vertebrates, the Wnt-signalling pathway yet remains to be fully elucidated^{1–3}. A better understanding is required not only from a fundamental biological point of view, but also because it can be important for developing drugs and medical treatments of Wnt associated diseases such as bone diseases and colorectal cancer (reviewed by^{4,5}).

The model worm *Caenorhabditis elegans*, a widely studied human model species, has the canonical Wnt-signalling pathway and a variation on this pathway, the asymmetrical cell division pathway. The Wnt pathway has five known Wnt genes: *mom-2*, *cwn-1*, *cwn-2*, *lin-44* and *egl-20*⁶. In the canonical Wnt pathway, the cellular abundance of free β -catenin is controlled by a protein destruction complex which targets free β -catenin for proteasomal degradation. Activation of canonical Wnt-signalling, whereby Wnt binds to a Frizzled/LRP co-receptor, inactivates the destruction complex leading to accumulation of free β -catenin which then functions as a nuclear transcriptional activator. In *C. elegans* four distinct β -catenins have been identified. *Bar-1* is part of the evolutionarily most conserved pathway^{7–9}, whereas *wrm-1*¹⁰, *hmp-2*¹¹, and *sys-1*¹² function in a variant of the Wnt pathway regulating asymmetrical cell divisions^{6,7}. These β -catenins all seem to play distinct roles in the worm¹³.

BAR-1 functions in the post-embryonic stage^{8,14,15} where it forms a transcription activation complex with the Wnt effector POP-1¹³, similar to the TCF/ β -catenin complex in flies and vertebrates¹⁶. BAR-1 is regulated by the axin-like protein PRY-1, the GSK3 β homolog SGG-1 and the APC-like protein APR-1^{17,18}. Among the processes influenced by BAR-1 are P12 cell fate specification^{14,19} and *mab-5* expression in the neuroblast QL^{15,20}. Moreover, BAR-1 is involved in vulval precursor cell specification in the early L1 stage of *C. elegans* through transcriptional activation of the Hox gene *lin-39*. In addition, BAR-1 plays a role in cell fate specification of the vulva during the early L3 stage⁸. The mutation used in this study, *bar-1(ga80)*, affects vulval precursor cell induction which results in an incomplete vulva, a protruding vulva (pvl) and egg-laying defects (egl)⁸.

Even though many developmental processes in which *bar-1* is involved are known, the effect of *bar-1* on gene expression is poorly understood. We studied gene expression patterns during the fourth larval stage (L4) of the



worm strain EW15 carrying the β -catenin-loss-of-function point mutation *bar-1(ga80)*. This mutation causes a Glu to Stop codon change at amino acid 97 of the predicted BAR-1 protein. By analysing the transcriptome and developmental phenotyping we show that *bar-1(ga80)* impairs developmental timing. Moreover we found that without a functioning *bar-1* ~ 7,500 genes were affected. Our results further suggest that the loss of *bar-1* is partially compensated by redundancy in the Wnt-signalling pathway, pointing towards a feedback mechanism between β -catenin activation and the expression levels of Wnt-signalling pathway encoding genes.

Methods

Strains. The following strains were used: EW15 (Bristol N2 strain, carrying the mutation *bar-1(ga80)*) and wild type Bristol N2. Upon arrival in the lab EW15 was outcrossed at least 4 times. Worms were kept in maintenance at 12°C and before experiments started, populations were cleared of males and grown at 20°C until all worms were gravid. All experiments were conducted at 20°C.

Microarray experiment. Strains were stage synchronized by bleaching²¹, then grown on 9 cm NGM Petri dishes seeded with *E. coli* OP50. Worms were rinsed of the plates with M9 buffer 48 hours after synchronization, snap-frozen in liquid nitrogen and stored at -80°C before performing gene expression profiling using microarray analysis. The experiment was performed in three independent duplicates.

Egg laying experiment. Strains were synchronised by bleaching, after which approximately 200 eggs from N2 or 300 eggs from EW15 were transferred to a fresh 9 cm NGM dish containing *E. coli* OP50. Starting at 58 hours after bleaching, the production of eggs by the adult worms was observed. Eggs on the plate were scored as: 0 (no eggs), 1 (first 1–100 eggs, first worms have started laying), 2 (100–200 eggs, multiple worms are laying eggs), 3 (>200, most worms are laying eggs, first egg clusters appear) or 4 (many eggs and the first eggs are hatching). The scoring was done every hour. The experiment was performed in three independent duplicates.

Egg hatching experiment. Strains were synchronised by bleaching, after which as many eggs as possible (up to 700 eggs) were placed on a fresh 9 cm NGM dish containing *E. coli* OP50. Observations started immediately after bleaching, for every 30 minutes. Once an egg hatched, the L1 larvae was picked and counted. This was continued until all eggs hatched. The experiment was performed three times.

Microarray sample preparation, scanning and normalization. mRNA isolation was performed using the RNeasy Micro Kit from Qiagen (Hilden, Germany), following the 'Purification of Total RNA from Animal and Human Tissues' -protocol provided with the kit. After this, the 'Two-Color Microarray-Based Gene Expression Analysis; Low Input Quick Amp Labeling' -protocol, version 6.0 from Agilent (Agilent Technologies, Santa Clara, CA, USA) was followed, starting from step 5. The microarrays used were *C. elegans* (V2) Gene Expression Microarray 4 × 44K slides, manufactured by Agilent. Input of total RNA was approximately 200 ng for each replicate. Three independent duplicates per strain were measured. The microarrays were scanned using an Agilent High Resolution C Scanner, using the settings as recommended in the above mentioned manual. Data was extracted with the Agilent Feature Extraction Software version 10.5, following manufacturers' guidelines. For normalization the Limma package for the "R" environment (version 2.13.1 x 64) was used. No background correction of the RNA-array data was performed as recommended by²². For within-array normalization of the RNA-array data the Loess method was used and for between-array normalization the Quantile method was used. The obtained log2 normalized intensities (single channel data) were used for further analysis.

Statistical analyses. All statistical analyses were performed using the statistical programming language "R" (version 2.13.1 x 64). A linear model was used to determine the effect and significance of the genotype on the expression levels (probe intensity ~ genotype + error). Using permutations of the original data in the same linear model, we determined thresholds adjusted for multiple testing (FDR 0.05: $-\log_{10}(p) > 2$; FDR 0.01: $-\log_{10}(p) > 3$). To correct for the developmental difference between *bar-1(ga80)* and N2 we used the developmental gene expression data from Snoek *et al.* (2014)²³ together with the gene expression data generated for this study (*bar-1(ga80)* vs. N2) in one linear model (probe intensity ~ sample age + genotype + error). The intensities were corrected for batch effect and for sample age we used an age of 44 hours for the *bar-1(ga80)* samples (as estimated), and 48 hours for the N2 samples. For the samples from Snoek *et al.* (2014) their original ages were used (44 to 58 hours after synchronisation). Genes with $p > 0.05$ for the "sample age" were selected as genes without a developmental effect. Genes with for which the "sample age" effect was opposite to the *bar-1* effect were selected as genes with "effect which was opposite of what one would expect in a relatively slower developed *bar-1(ga80)* mutant".

Enrichment tests were done using a hyper geometric test on the genes with a significant *bar-1(ga80)* effect ($-\log_{10}(p) > 2$), excluding those with a developmental effect (unless stated otherwise). eQTL enrichments were done by selecting the genes

with a significant ($-\log_{10}(p) > 3$) linkage to each locus and comparing those against the genes affected by the mutant²⁴.

Datasets used. The GO-annotation, anatomy terms, protein domains and gene classes were obtained via Wormmart (www.caprica.caltech.edu:9002/biomart/martview/) of the WS220 wormbase release. Genes from Wormbook chapters were obtained from the 2012 version of Wormbook (www.wormbook.org). Expression QTLs (eQTLs) were obtained from WormQTL (www.wormqtl.org;^{25,26,49} using the data from^{27–29}). Transcription factor binding sites were obtained from³⁰ Binding sites from DAF-16 were obtained from modENCODE release #32 (www.modencode.org;³¹), and mapped to transcription start sites according to Tepper *et al.* (2013)³². KEGG pathways were obtained from Release 65.0 of the Kyoto Encyclopedia of Genes and Genomes (www.genome.jp/kegg/).

Network visualization. The network of the transcription factors and their targets was visualized using Cytoscape (version 2.8.2)³³.

Data storage. All data was stored in WormQTL (www.wormqtl.org)^{25,26,49}.

Results and discussion

***bar-1(ga80)* affects gene expression and slows development.** We compared the transcriptomes of worms (age of 48 h) of N2 to the *bar-1(ga80)* mutant strain of the same age by microarray analysis. Of the 20,887 genes that were tested on the microarray, 5,772 genes were differentially expressed ($-\log_{10}(p) > 2.0$ at FDR = 0.05). In *bar-1(ga80)*, 51% was down-regulated (2,927 genes), and 49% was up-regulated (2,855 genes) compared to N2. During the initial analyses, we noticed that many of the differentially expressed genes were related to development, for example genes encoding for collagens and vitellogenins. Recently we reported that genome wide gene expression can rapidly and massively change during the L4 stage²³. To test for the developmental difference within the L4 stage, we compared the differentially expressed genes between N2 and *bar-1(ga80)* with the set of genes reported by Snoek *et al.* (2014)²³ (Figure 1). The differentially expressed genes between *bar-1(ga80)* and N2 were enriched for genes changing during L4 development (hypergeometric test, $p < 1 \times 10^{-200}$). To exactly pinpoint the developmental delay, we used the expression levels which have a linear correlation with L4 developmental timing to estimate the developmental age of the *bar-1(ga80)* and N2 samples. Even though all RNA samples from both genotypes were taken at 48 hours after synchronisation we found that the *bar-1(ga80)* worms developed more slowly ($44.4 \text{ h} \pm 0.9$) than the N2 samples ($47.7 \text{ h} \pm 0.8$) (two-sided t-test, $p = 6 \times 10^{-5}$) (Supplementary figure

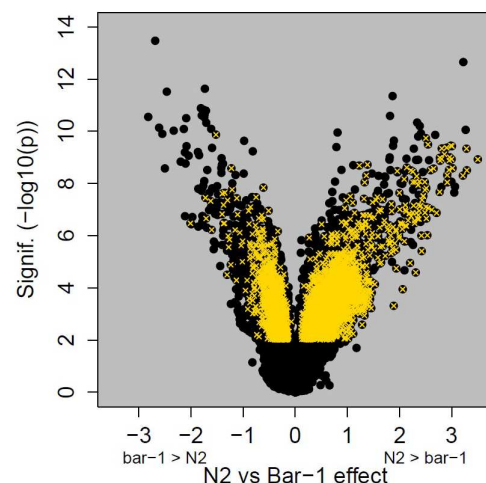


Figure 1 | Genes affected by *bar-1* and development. Volcano plot showing the effects and significance of the transcriptome comparison between *bar-1(ga80)* and N2. The black dots represent the spots on the array, the log2 effect between N2 and *bar-1(ga80)* is shown versus the LOD score. The yellow x indicate spots of genes affected by developmental effects during L4 development²³.

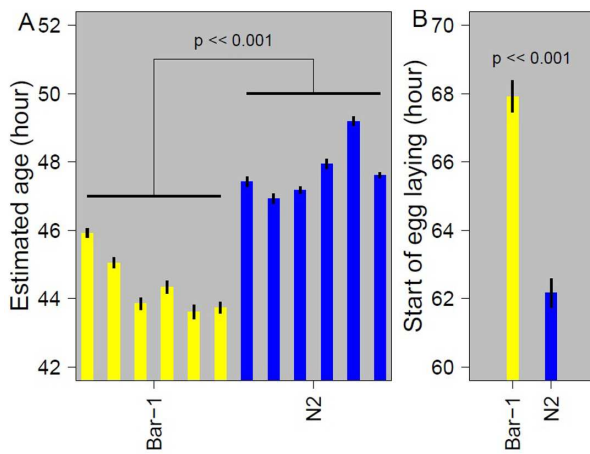


Figure 2 | Age-estimation and start of egg-laying in N2 and *bar-1(ga80)*. A) Age estimates for N2 (blue) and *bar-1(ga80)* (yellow), based on linearly differentially expressed genes during L4 development²³. The *bar-1(ga80)* mutant is estimated significantly younger than N2 ($p < 1 \times 10^{-4}$). B) Start of egg-laying in hours after synchronization for N2 (blue) and *bar-1(ga80)*. Again, the difference between the two strains is significant ($p < 1 \times 10^{-5}$).

A and Figure 2A) indicating a developmental delay of 3.3 hours after 48 hours.

To confirm this discrepancy we recorded the time until the first eggs were laid in N2 and *bar-1(ga80)*. N2 started laying eggs at ~62 hours and *bar-1(ga80)* started laying eggs at ~68 hours (two-sided t-test, $p = 4 \times 10^{-6}$) (Figure 2B). To investigate whether the delay was caused in part by delayed hatching or slow embryonic development, the time until hatching after synchronization was determined. No difference was found between N2 and *bar-1(ga80)* in time from synchronising the eggs and hatching of those eggs (Supplementary figure B).

The developmental delay of *bar-1(ga80)* increased over time (0 h at 0 h, -3.3 h at 48 h and 6 h at 62 hour). This implies that the mutation affected the entire developmental period from egg to adult. Our results show that *bar-1(ga80)* does not affect a single developmental stage, because the developmental difference between *bar-1(ga80)* and N2 would remain constant during the subsequent stages.

Analysis incorporating developmental effects. To exclude the effects of the developmental delay of *bar-1(ga80)* from other effects of *bar-1(ga80)* on gene expression, we included the transcriptional effects during L4 development in the analysis (Figure 3). Here we found 7,557 (FDR = 0.05) genes to be affected by the *bar-1(ga80)* mutation either with or without a developmental effect. Of these genes, 3,920 were up-regulated and 3,637 were down-regulated in the *bar-1(ga80)* mutant (Supplementary figure C).

As developmental effects were very strong and affected many genes^{23,34}, we selected those genes that did not have a developmental effect ($P < 0.05$) or an effect which was opposite of what one would expect in a relatively slower developed *bar-1(ga80)* mutant (Supplemental figure C). We also selected on effect size (>0.5 or <-0.5) which resulted in 710 down- and 425 up-regulated genes compared to N2 (FDR = 0.05; Supplement Table 1).

BAR-1 strongly affects collagens and hedge-hog signalling. The set of genes down-regulated in *bar-1(ga80)* compared to N2 (Supplement Table 1) contains many non-annotated genes. These genes could complement the genes with known functions, but could also constitute new functions. Furthermore genes like *mai-1*, *dao-4*, *pho-11*, *sta-2*, *plc-2*, *pes-8*, *cnp-2*, *hmit-1.1*, *hmit-1.2*, *gcy-32*, *nlp-23* and *fkb-5* have a strongly reduced expression in *bar-1(ga80)*. These genes

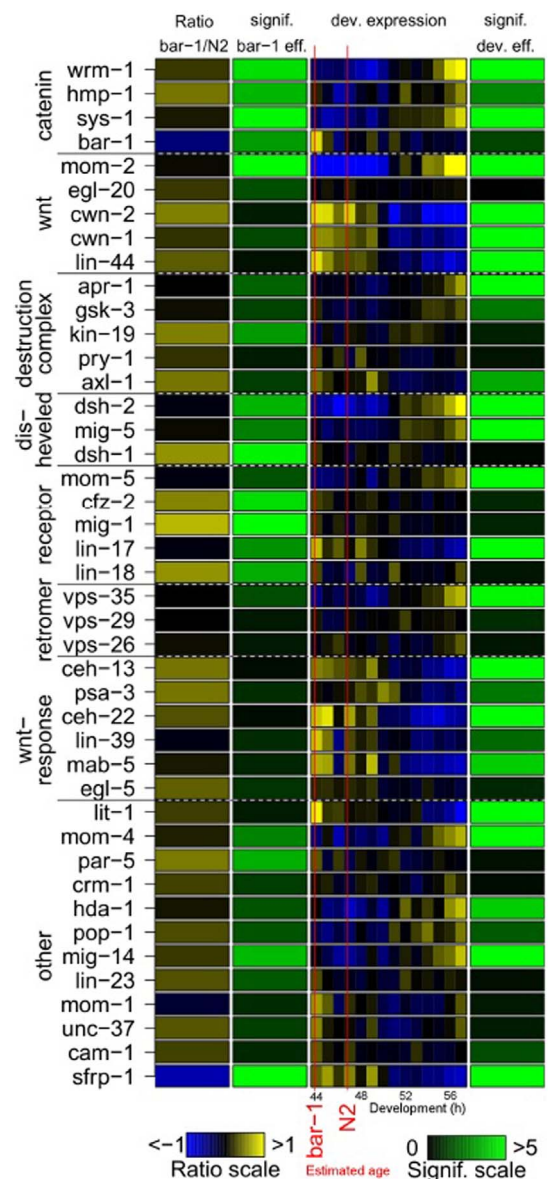


Figure 3 | Effects on Wnt pathway genes. Members of the Wnt pathway are shown by their function. The first column shows the log2-ratio between the *bar-1* mutant and N2, blue indicates lower in *bar-1*, yellow higher in *bar-1*. The second column shows the significance ($-\log_{10}(p)$) of the *bar-1* effect. The third column shows the log2-ratio during L4 stage (44 to 58 hours at 20°C)²³. Two red lines indicate the relative development of the *bar-1* and N2 samples taken at 48 hours post hatching. Last column shows the significance ($-\log_{10}(p)$) of the developmental effect.

might function together with the strongly down-regulated groups of genes, like collagens (*rol-1*, *bli-2*, *bli-1*, *dpy-3*, *lon-3*), col-type genes (*col-175*, -38, -71, -120, -40, -49, -138, -110, -97, -79, -70) or other cuticle related components (*cutl-18*, *cutl-28*, *mlt-18*, *mltn-12*, *nas-27* and *gly-1*). Components of hedge-hog (hh) signalling were also much lower expressed in *bar-1(ga80)* such as: the warthog genes *wrt-6* and *wrt-4*; groundhog-like genes *grl-15*, *grl-5* and *grl-14*; hedgehog-like genes, *hog-1*, *grd-2*, *grd-1* and *grd-12*. This shows that hh-signalling is affected by *bar-1(ga80)* mutation. Taken together, BAR-1 activity is most likely required for activation of collagens and other cuticle genes as well as genes involved in hedge-hog signalling.

Expression of Wnt-signalling components. The expression of most Wnt-signalling components changed during development and were



affected by *bar-1(ga80)* mutation (Figure 3) (core-Wnt pathway genes selected by⁶). All four β -catenins were differentially expressed in *bar-1(ga80)*. Expression of *bar-1* was lower whereas *wrm-1*, *hmp-1* and *sys-1* all showed a slight increase in expression (see 4 upper blocks in the first column of Figure 3). In N2, these three β -catenins showed increased expression levels during L4 development whereas expression of *bar-1* hardly changed throughout the L4 stage in N2 ($p = 0.042$; see 4 upper blocks in the third column of Figure 3).

Of the five Wnt genes in *C. elegans*, only *mom-2* was higher expressed in *bar-1(ga80)*. For the other Wnt genes no significant effect was found. Of the Wnt genes, only *mom-2* expression increased during L4 development, whereas expression of *cwn-1*, *cwn-2* and *lin-44* decreased ($p < 0.001$ in all cases). *Egl-20* was not differentially expressed throughout L4 and was also not affected by the *bar-1* mutation.

The members of the destruction complex showed no or only minor expression differences between *bar-1(ga80)* and N2 during development. One of the dishevelled genes, *dsh-1*, was affected by the *bar-1* mutation. This gene was higher expressed in *bar-1(ga80)*. It was also the only gene of the three dishevelled genes that did not show a change in expression.

The Wnt receptors *cfz-2*, *mig-1* and *lin-18* (*ryk/derailed*) were higher expressed in *bar-1(ga80)*, and their expression did not change during development. The two other Wnt-receptors were slightly affected by development. Expression of *mom-5* increased during L4 development whereas *lin-17* expression decreased. Of the other genes, *sfrp-1*, an extracellular active Wnt-inhibitor³⁵, had a lower expression in the *bar-1* mutant compared to N2. The expression of *sfrp-1* decreased during L4 development in N2. Even though *bar-1(ga80)* showed a developmental delay, the expression of *sfrp-1* decreased compared to N2. Some of the transcription factors known to be involved in the Wnt-pathway, like EOR-1³⁶ and EGL-27³⁷ (or associated with the Wnt pathway, like SKN-1³⁸), were up regulated, but their targets were not enriched for in the differentially expressed genes set (hypergeometric test, $p > 0.1$).

To summarize, the up-regulation of the other three β -catenins in *bar-1(ga80)* probably compensates for the loss of a functional BAR-1 (Supplement figure C). Intriguingly, not only the β -catenins were higher expressed, but also the Wnt-receptors. Furthermore, the *sfrp-1* gene was down-regulated. Thus, a lack of *bar-1* also affects the Wnt-signalling pathway upstream, which could point to a feedback-mechanism. As the Wnt encoding gene *mom-2*, the Wnt-receptors *cfz-2*, *mig-1* and *lin-18* and the dishevelled gene *dsh-1* were also up-regulated, our results imply that the Wnt- signalling pathway itself was activated following the knock-down of *bar-1*.

Natural genetic variation in Wnt-pathway genes. All genes part of, or associated with, the Wnt-pathway (Figure 3) are polymorphic across many other *C. elegans* wild type strains^{39–41}. Between the two most frequently studied wild types N2 and CB4856 these polymorphisms lead to an amino acid change in almost 50% of the proteins (Supplement text 1). Furthermore enrichments of expression Quantitative Trait Loci (eQTL) of genes with affected transcript levels by *bar-1(ga80)* suggest that polymorphic loci between CB4856 and N2 downstream of or modulated by *bar-1* and Wnt-signalling might be present (Supplement text 1). This indicates that the Wnt-signalling pathway is genetically buffered⁴² and the associated genes are possibly co-evolving.

Biological processes affected by *bar-1(ga80)*. To investigate which processes were affected by the *bar-1(ga80)* mutation, we tested enrichment of mutation-affected genes in GO-, KEGG-, Anatomy-, Wormbook-, Gene class- and Protein domain annotations. To distinguish between *bar-1* and developmental effects we excluded all the *bar-1(ga80)* affected genes with a developmental effect from the set of genes used for enrichment analysis (Supplementary

Table 2). The results of the complete set of *bar-1(ga80)* affected genes including those with a developmental effect can also be found in Supplementary Table 2.

Genes lower expressed in *bar-1(ga80)* are enriched with genes involved in cuticle constituents ($p < 1*10^{-4}$), proteolysis ($p < 1*10^{-10}$) and the proteasome core complex ($p < 1*10^{-10}$). Whereas the proteolysis and proteasome core complex genes overlapped, they did not overlap with the cuticle constituent genes. Furthermore protein degradation related enrichments were reflected in the multiple categories tested, implying that protein degradation/turn-over might be reduced. Thus, *bar-1(ga80)* affects protein degradation, possibly reflecting the transition of the cell from one state into another.

The group of genes expressed higher in *bar-1(ga80)* consisted of a more diverse set of genes. These genes were especially related to transcriptional regulation, as shown by an enrichment of the GO-terms regulation of transcription ($p < 1*10^{-8}$), sequence specific DNA binding ($p < 1*10^{-8}$), transcription factor activity ($p < 1*10^{-8}$) and nucleus ($p < 1*10^{-7}$). Some indications were found that the Ras-pathway was affected because transcription factors known to be linked to the Ras-pathway were up-regulated, like the RAS inhibitors MDL-1⁴³ and LIN-15B⁴⁴. The activation of the Ras pathway is further shown by the strong up regulation of *cav-1* in the *bar-1(ga80)* mutant.

Furthermore, also neuron-related terms were represented, as shown by enrichments of the GO-term axon ($p < 1*10^{-6}$), synapse ($p < 1*10^{-4}$) and in the anatomy terms where the three most significantly enriched groups were neuronal ($p < 1*10^{-10}$). The enrichment of these neuronal genes can point in the direction of the aberrant neuron migration that is observed in *bar-1(ga80)*¹⁵, the mutation might affect neuropeptide signalling.

***bar-1(ga80)* transcription patterns suggest DAF-16 activation.**

Since enrichment in transcriptional regulation was detected, we used the modENCODE^{30,31} set of ChIP-seq determined binding sites to search for enrichment of binding sites for transcription factors. We found that the genes higher expressed in *bar-1(ga80)* were enriched for binding-sites of transcription factors PHA-4, MDL-1, ELT-3 and PQM-1 (hypergeometric test, $P < 1*10^{-2}$). These transcription factors were up-regulated in the *bar-1(ga80)* mutant, except for *elt-3* (Figure 4A). Together with the enrichment found for the binding sites, this indicates that the absence of the β -catenin BAR-1 results in an activation of transcription factors, possibly as a compensatory response. The four transcription factors for which enrichments have been found share binding sites for many of the genes. Over 50% of the up-regulated genes in the transcriptional network were associated with more than one of these four transcription factors (Figure 4B). Furthermore, PQM-1 and MDL-1 also bind near the transcription starting site of PHA-4 and ELT-3 (Supplementary figure D)³⁰. This indicates that it is likely that PQM-1 or MDL-1 is involved in the transcriptional activation observed in *bar-1(ga80)*.

Three of these transcription factors: PQM-1³², MDL-1⁴⁵, and ELT-3⁴⁶, have been associated with the insulin/IGF-1 signalling pathway and longevity. However, for ELT-3 this relation is debated in more recent literature⁴⁷. Furthermore, PQM-1 is also identified as a promoter of growth, development and reproduction³². PQM-1 has an antagonistic interaction with DAF-16, where nuclear translocation of PQM-1 (promoted by DAF-2) results in depletion of DAF-16 from the nucleus (and vice-versa). Tepper *et al.* identified genes regulated by PQM-1 (referred to as class II genes), and genes regulated by DAF-16 (referred to as class I genes). It is also shown that some of the class I genes are also regulated by PQM-1³². We tested expression of these genes in the *bar-1(ga80)* versus N2 and found that the class I genes were enriched for in the up-regulated genes (hypergeometric test, $p < 1*10^{-22}$) and slightly but significantly

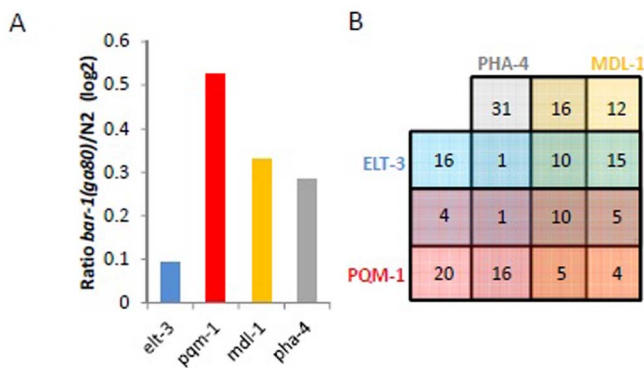


Figure 4 | Transcription factor activity and targets in up-regulated genes. (A) shows the transcript abundance of the four transcription factors enriched for targets among the up-regulated genes in *bar-1(ga80)*. The fold-change in *bar-1/N2* is shown. The levels of *pqm-1*, *pha-4*, and *mdl-1* are significantly higher in *bar-1(ga80)* (linear model, $p < 0.01$), whereas this is not the case for *elt-3* (linear model, $p = 0.158$). (B) A Venn-diagram of the up-regulated genes associated with the four enriched transcription factors. There is a high level of overlap between the associations as $>50\%$ of the targets are associated with multiple transcription factors.

up-regulated in *bar-1(ga80)* (two-sided t-test, $p < 1 \times 10^{-8}$). Moreover, the specific PQM-1 targets were enriched for in the down-regulated genes (hypergeometric test, $p < 1 \times 10^{-2}$) and were slightly down-regulated (two-sided t-test, $p < 1 \times 10^{-5}$), see also Supplementary figure E and Supplementary Table 3. We also analysed DAF-16 ChIP-seq data³¹, and found that the genes up-regulated in *bar-1(ga80)* were enriched for DAF-16 targeted genes (124 out of 425 up-regulated genes, hypergeometric test, $p < 1 \times 10^{-3}$). Based on these results we hypothesize that loss of function of *bar-1(ga80)* leads to induction of a stress response reflecting DAF-16 activation, causing delayed development of the worms.

Conclusion

We studied gene expression patterns during the fourth larval stage (L4) of the strain EW15 carrying the β -catenin-loss-of-function point mutation *bar-1(ga80)* causing a Glu to Stop codon change at amino acid 97 of the predicted BAR-1 protein. To untangle the developmental effects from the effects of the *bar-1* mutation, we used a time-series dataset²³. We showed that *bar-1(ga80)* results in a slower development, as these worms take on average $\sim 10\%$ more

time to develop than Bristol N2. Using the transcriptome to estimate the age of the worms, we found that 48 hours after synchronization, *bar-1(ga80)* worms are transcriptionally most similar to N2 worms at 44–45 hours after synchronization²³. By measuring the time that egg-deposit starts in *bar-1(ga80)* and Bristol N2, we confirmed this developmental delay. To our knowledge, this has not yet been reported for *bar-1*.

Analysis of the Wnt-pathway showed that a non-functional *bar-1* causes up-regulation of Wnt-signalling components, *mom-2*, *cfz-2*, *mig-1*, *lin-18*, *dsh-1*, *mom-5* and *lin-17*. Together with the down-regulation of Wnt-inhibitor *sfrp-1* this indicates hyper-activation of the Wnt-signalling pathway, suggesting a compensatory mechanism (Figure 5). This is further shown by the modest up-regulation of the other β -catenins, *wrm-1*, *hmp-2* and *sys-1* which all have the potential to substitute for *bar-1* in transcriptional activation^{12,48}.

Analysis of the genes affected by *bar-1(ga80)* showed that genes up-regulated in *bar-1(ga80)* are enriched for transcription factor- as well as histone- binding sites and for processes like chromosome rearrangement, chromatin factors and neurogenesis. The down-regulated genes were enriched for cuticle components and hh-signalling pathway genes, suggesting *bar-1* directly affects these processes. We found that the transcriptional response induced in *bar-1(ga80)* reflects DAF-16 activation (Figure 5). This also corresponds with the developmental delay we measured. We propose that loss of *bar-1* results in a compensatory/feedback response on the transcriptional level, leading to Wnt-pathway and DAF-16 activation.

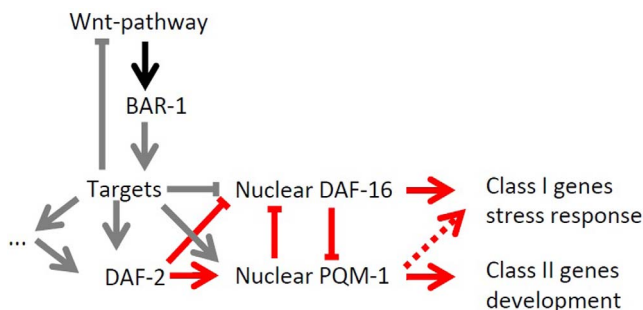


Figure 5 | A model for the *bar-1lof* effects. This model incorporates the findings in this paper (grey) with what is known about the Wnt-pathway regarding to BAR-1 (black)⁶ and findings reported about DAF-16 and PQM-1 (red)³². It is proposed that (transcriptional) activity of the β -catenin *bar-1* results in a feedback loop, de-activating the Wnt-pathway. Furthermore BAR-1 activity is needed for a correct developmental program, where *bar-1lof* shows indications of a DAF-16-mediated stress response. The exact level of interaction between BAR-1 and the insulin pathway remains to be elucidated.

- Buechling, T. & Boutros, M. Wnt signaling signaling at and above the receptor level. *Curr. Top. Dev. Biol.* **97**, 21–53, doi:10.1016/B978-0-12-385975-4.00008-5 (2011).
- Moon, R. T., Bowerman, B., Boutros, M. & Perrimon, N. The promise and perils of Wnt signaling through beta-catenin. *Science* **296**, 1644–1646, doi:10.1126/science.1071549 (2002).
- Niehrs, C. The complex world of WNT receptor signalling. *Nat. Rev. Molec. Cell Biol.* **13**, 767–779, doi:10.1038/nrm3470 (2012).
- Clevers, H. & Nusse, R. Wnt/beta-catenin signaling and disease. *Cell* **149**, 1192–1205, doi:10.1016/j.cell.2012.05.012 (2012).
- MacDonald, B. T., Tamai, K. & He, X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev. Cell* **17**, 9–26, doi:10.1016/j.devcel.2009.06.016 (2009).
- Jackson, B. M. & Eisenmann, D. M. Beta-catenin-dependent Wnt signaling in *C. elegans*: teaching an old dog a new trick. *Cold Spring Harbor Perspect. Biol.* **4**, a007948, doi:10.1101/cshperspect.a007948 (2012).
- Eisenmann, D. M. Wnt signaling. *WormBook: the online review of C. elegans biology*, 1–17, doi:10.1895/wormbook.1.7.1 (2005).
- Eisenmann, D. M., Maloof, J. N., Simske, J. S., Kenyon, C. & Kim, S. K. The beta-catenin homolog BAR-1 and LET-60 Ras coordinately regulate the Hox gene *lin-39* during *Caenorhabditis elegans* vulval development. *Dev.* **125**, 3667–3680 (1998).
- Gleason, J. E., Szyleyko, E. A. & Eisenmann, D. M. Multiple redundant Wnt signaling components function in two processes during *C. elegans* vulval development. *Dev. Biol.* **298**, 442–457, doi:10.1016/j.ydbio.2006.06.050 (2006).
- Rocheleau, C. E. *et al.* Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. *Cell* **90**, 707–716 (1997).
- Costa, M. *et al.* A putative catenin-cadherin system mediates morphogenesis of the *Caenorhabditis elegans* embryo. *J. Cell. Biol.* **141**, 297–308 (1998).
- Kidd, A. R., 3rd, Miskowski, J. A., Siegfried, K. R., Sawa, H. & Kimble, J. A beta-catenin identified by functional rather than sequence criteria and its role in Wnt/MAPK signaling. *Cell* **121**, 761–772, doi:10.1016/j.cell.2005.03.029 (2005).
- Korswagen, H. C., Herman, M. A. & Clevers, H. C. Distinct beta-catenins mediate adhesion and signalling functions in *C. elegans*. *Nature* **406**, 527–532, doi:10.1038/35020099 (2000).
- Eisenmann, D. M. & Kim, S. K. Protruding vulva mutants identify novel loci and Wnt signaling factors that function during *Caenorhabditis elegans* vulva development. *Genet.* **156**, 1097–1116 (2000).
- Maloof, J. N., Whangbo, J., Harris, J. M., Jongeward, G. D. & Kenyon, C. A Wnt signaling pathway controls hox gene expression and neuroblast migration in *C. elegans*. *Dev.* **126**, 37–49 (1999).
- van de Wetering, M. *et al.* Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell* **88**, 789–799 (1997).
- Gleason, J. E., Korswagen, H. C. & Eisenmann, D. M. Activation of Wnt signaling bypasses the requirement for RTK/Ras signaling during *C. elegans* vulval induction. *Gen. Dev.* **16**, 1281–1290, doi:10.1101/gad.981602 (2002).



18. Korswagen, H. C. *et al.* The Axin-like protein PRY-1 is a negative regulator of a canonical Wnt pathway in *C. elegans*. *Gen. Dev.* **16**, 1291–1302, doi:10.1101/gad.981802 (2002).
19. Jiang, L. I. & Sternberg, P. W. Interactions of EGF, Wnt and HOM-C genes specify the P12 neuroectoblast fate in *C. elegans*. *Dev.* **125**, 2337–2347 (1998).
20. Whangbo, J. & Kenyon, C. A Wnt signaling system that specifies two patterns of cell migration in *C. elegans*. *Mol. Cell.* **4**, 851–858 (1999).
21. Sulston, J. E. & Hodgkin, J. in *The nematode Caenorhabditis elegans* (ed Wood, W. B.) 587–606 (Cold Spring Harbor Laboratory 1988).
22. Zahurak, M. *et al.* Pre-processing Agilent microarray data. *BMC Bioinf.* **8**, 142, doi:10.1186/1471-2105-8-142 (2007).
23. Snoek, L. B. *et al.* A rapid and massive gene expression shift marking adolescent transition in *C. elegans*. *Sci. Rep.* **4**, 3912, doi:10.1038/srep03912 (2014).
24. Terpstra, I. R., Snoek, L. B., Keurentjes, J. J. B., Peeters, A. J. M. & Van den Ackerveken, G. Regulatory Network Identification by Genetical Genomics: Signaling Downstream of the Arabidopsis Receptor-Like Kinase ERECTA. *Plant. Physiol.* **154**, 1067–1078, doi:DOI 10.1104/pp.110.159996 (2010).
25. Snoek, L. B. *et al.* WormQTL—public archive and analysis web portal for natural variation data in *Caenorhabditis* spp. *Nucl. Ac. Res.* **41**, D738–743, doi:10.1093/nar/gks1124 (2013).
26. van der Velde, K. J. *et al.* WormQTLHD—a web database for linking human disease to natural variation data in *C. elegans*. *Nucl. Ac. Res.* **42**, D794–801, doi:10.1093/nar/gkt1044 (2014).
27. Vinuela, A., Snoek, L. B., Riksen, J. A. G. & Kammenga, J. E. Genome-wide gene expression regulation as a function of genotype and age in *C. elegans*. *Genome Res.* **20**, 929–937, doi:DOI 10.1101/gr.102160.109 (2010).
28. Vinuela, A., Snoek, L. B., Riksen, J. A. G. & Kammenga, J. E. Aging Uncouples Heritability and Expression-QTL in *Caenorhabditis elegans*. *G3-Genes Genom. Genet.* **2**, 597–605, doi:DOI 10.1534/g3.112.002212 (2012).
29. Rockman, M. V., Skrovanek, S. S. & Kruglyak, L. Selection at linked sites shapes heritable phenotypic variation in *C. elegans*. *Science* **330**, 372–376, doi:10.1126/science.1194208 (2010).
30. Niu, W. *et al.* Diverse transcription factor binding features revealed by genome-wide ChIP-seq in *C. elegans*. *Genome Res.* **21**, 245–254, doi:DOI 10.1101/gr.114587.110 (2011).
31. Gerstein, M. B. *et al.* Integrative analysis of the *Caenorhabditis elegans* genome by the modENCODE project. *Science* **330**, 1775–1787, doi:10.1126/science.1196914 (2010).
32. Tepper, R. G. *et al.* PQM-1 Complements DAF-16 as a Key Transcriptional Regulator of DAF-2-Mediated Development and Longevity. *Cell* **154**, 676–690, doi:DOI 10.1016/j.cell.2013.07.006 (2013).
33. Shannon, P. *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498–2504, doi:10.1101/gr.1239303 (2003).
34. Kim, D. H., Grun, D. & van Oudenaarden, A. Dampening of expression oscillations by synchronous regulation of a microRNA and its target. *Nat. Genet.* **45**, 1337–+, doi:DOI 10.1038/Ng.2763 (2013).
35. Harterink, M. *et al.* Neuroblast migration along the anteroposterior axis of *C. elegans* is controlled by opposing gradients of Wnts and a secreted Frizzled-related protein. *Dev.* **138**, 2915–2924, doi:DOI 10.1242/Dev.064733 (2011).
36. Howard, R. M. & Sundaram, M. V. C. elegans EOR-1/PLZF and EOR-2 positively regulate Ras and Wnt signaling and function redundantly with LIN-25 and the SUR-2 Mediator component. *Gen. Dev.* **16**, 1815–1827, doi:10.1101/gad.998402 (2002).
37. Herman, M. A. *et al.* EGL-27 is similar to a metastasis-associated factor and controls cell polarity and cell migration in *C. elegans*. *Dev.* **126**, 1055–1064 (1999).
38. Maduro, M. F., Kasmir, J. J., Zhu, J. W. & Rothman, J. H. The Wnt effector POP-1 and the PAL-1/Caudal homeoprotein collaborate with SKN-1 to activate *C. elegans* endoderm development. *Dev. Biol.* **285**, 510–523, doi:DOI 10.1016/j.ydbio.2005.06.022 (2005).
39. Andersen, E. C. *et al.* Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. *Nat. Genet.* **44**, 285–290, doi:10.1038/ng.1050 (2012).
40. Thompson, O. *et al.* The million mutation project: a new approach to genetics in *Caenorhabditis elegans*. *Genome Res.* **23**, 1749–1762, doi:10.1101/gr.157651.113 (2013).
41. Volkers, R. J. *et al.* Gene-environment and protein-degradation signatures characterize genomic and phenotypic diversity in wild *Caenorhabditis elegans* populations. *BMC Biol.* **11**, 93, doi:10.1186/1741-7007-11-93 (2013).
42. Felix, M. A. & Barkoulas, M. Robustness and flexibility in nematode vulva development. *Trends Genet.* **28**, 185–195, doi:10.1016/j.tig.2012.01.002 (2012).
43. Yuan, J., Tirabassi, R. S., Bush, A. B. & Cole, M. D. The *C. elegans* MDL-1 and MXL-1 proteins can functionally substitute for vertebrate MAD and MAX. *Oncog.* **17**, 1109–1118, doi:DOI 10.1038/sj.onc.1202036 (1998).
44. Clark, S. G., Lu, X. W. & Horvitz, H. R. The *Caenorhabditis-Elegans* Locus Lin-15, a Negative Regulator of a Tyrosine Kinase Signaling Pathway, Encodes 2 Different Proteins. *Genet.* **137**, 987–997 (1994).
45. Ackerman, D. & Gems, D. Insulin/IGF-1 and Hypoxia Signaling Act in Concert to Regulate Iron Homeostasis in *Caenorhabditis elegans*. *PLoS Genet.* **8**, doi:ARTN e1002498 DOI 10.1371/journal.pgen.1002498 (2012).
46. Budovskaya, Y. V. *et al.* An elt-3/elt-5/elt-6 GATA transcription circuit guides aging in *C. elegans*. *Cell* **134**, 291–303, doi:DOI 10.1016/j.cell.2008.05.044 (2008).
47. Tonsaker, T., Pratt, R. M. & McGhee, J. D. Re-evaluating the role of ELT-3 in a GATA transcription factor circuit proposed to guide aging in *C. elegans*. *Mech. Ageing. Dev.* **133**, 50–53, doi:DOI 10.1016/j.mad.2011.09.006 (2012).
48. Natarajan, L., Witwer, N. E. & Eisenmann, D. M. The divergent *Caenorhabditis elegans* beta-catenin proteins BAR-1, WRM-1 and HMP-2 make distinct protein interactions but retain functional redundancy in vivo. *Genet.* **159**, 159–172 (2001).
49. Snoek, L. B. *et al.* Worm variation made accessible: Take your shopping cart to store, link, and investigate! *Worm* **3**, e28357 (2014) <http://dx.doi.org/10.4161/worm.28357>.

Acknowledgments

LBS was funded by the ERASysbio-plus ZonMW project GRAPPLE (project nr. 90201066). MGS was supported by Graduate School Production Ecology & Resource Conservation. RJMV was funded by the NWO-ALW (project 855.01.151), TS, AH, JAGR and JEK were funded by PANACEA EU FP project contractnr. 222936. We thank Wormbase (www.wormbase.org) for being a rich and versatile source of information. We thank Morris Swertz and Joeri van der Velde for their help with making the data accessible through WormQTL. *Bar-1(ga80)* strain was kindly provided by S. Kim.

Author contributions

M.L.V.D.B., J.A.G.R., M.G.S., R.J.M.V. conducted the experiments. M.G.S., T.S., L.B.S. analysed the results, A.H., J.E.K., R.J.M.V., M.G.S. and L.B.S. wrote the manuscript.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: van der Bent, M.L. *et al.* Loss-of-function of β -catenin *bar-1* slows development and activates the Wnt pathway in *Caenorhabditis elegans*. *Sci. Rep.* **4**, 4926; DOI:10.1038/srep04926 (2014).



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. The images in this article are included in the article's Creative Commons license, unless indicated otherwise in the image credit; if the image is not included under the Creative Commons license, users will need to obtain permission from the license holder in order to reproduce the image. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/3.0/>